



Research Article

SCREENING OF IMMUNOMODULATORY EFFECT OF *SHATAPUTI ABHRAK BHASMA-AYURVEDA'S RASAYAN*

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ABSTRACT

Rasashastra, one of the branches of *Ayurveda*, deals with the pharmacological aspects of drugs, some of which have unique attributes. These unique attributes need to be validated and explored using the scientific and technological advances of today's world, which shall open up new avenues for drug processing, development and therapeutics in *Ayurveda*.

Abhrak Bhasma is one of the drugs of *Rasashastra* having some peculiar attributes. *Acharyas* have mentioned *Shataputi* and *Sahastaputi Abhrak Bhasma* which are indeed unique attributes of *Abhrak*. *Shataputi Abhrak Bhasma* is regarded as a *Rasayan*, as given in *Rasa Tarangini*, which says '*Abhrak Bhasma* given 20-100 *Putas* (incinerations) helps eliminate ailments, when given 100-1000 *Putas* acts like a *Rasayan*'.

Review of the current literature available on *Rasayanas* indicates that Anti-oxidant and Immunomodulation are the most studied activities of the *Rasayan* drugs. The effect Immunomodulation has on the human body can be compared to some extent with the effect of *Rasayan dravyas*, given in various Classical texts. In view of this correlation, *Shataputi Abhrak Bhasma* was subjected to *Invitro* screening to assess its Immunomodulatory effect using the Nitroblue Tetrazolium (NBT) assay. The results were self conclusive and indicated that *Shataputi Abhrak Bhasma* brings about stimulation of Leucocytes in concentration dependent manner. 5% and 10 % solutions of *Shataputi Abhrak Bhasma* stimulated 93% and 93.5% leucocytes respectively, which is an indicator of highly significant phagocytic activity. Thus, the study revalidates the reference of *Shataputi Abhrak Bhasma* as a *Rasayan* and hence also establishing it as an Immunomodulator.

KEYWORDS: *Rasayan*, *Shataputi Abhrak Bhasma*, Immunomodulatory Activity, Phagocytic activity, Nitroblue Tetrazolium Test, Leucocytes.

INTRODUCTION

A number of *Rasashastra* texts including *Rasa Tarangini* advocate the fact that *Abhrak* when given 20-100 *Putas*, helps in *Rognivrutti* (curing diseases) but when *Putas* are increased from 100-1000, it acts like a *Rasayan* (prevents disease).^[1] An attempt shall be made to understand and find a relation between the number of *Putas* and its preclinical efficacy with modern concept of *Rasayan* (Immunomodulation).

Concept of *Rasayan*

Rasayan is one of the eight branches of *Ayurveda*. The word *Rasayan* is made up of two words, '*Rasa*' and '*Ayan*'. The word *Rasa* has many meanings. It means *Shrangara*, *Visha*, *Virya*, *Guna*, *Raga*, *Drava* etc. *Ayan* means the pathway. Hence literally, *Rasayan* means the pathway to attain best quality of *Dhatus*. It helps maintain the health and also cures and prevents ailments.^[2]

Benefits of *Rasayan* Therapy^[3,4]

According to *Acharya Charak*, *Rasayan* therapy

- Enhances the intelligence, memory power, will power, body strength, skin lustre, sweetness of voice and physical strength.
- It nourishes the *Sapta dhatu* and thus prevents chronic degenerative changes and illness.
- *Rasayan* is thought to improve metabolic processes, which results in the best possible biotransformation and produce best quality body tissues, eradicate senility and thus help prevent diseases of the old age.
- It helps attain optimal physical strength and sharpness of sense organs.
- *Rasayan dravyas* have significant action on reproductive system and nourishes *Shukra dhatu*.

- *Rasayan* nourishes the whole body, helps maintain physiological functions at optimum level, thus also improves body's natural resistance against infections by increasing Immunity.
- *Rasayan* invigorates the body in general by sustaining the required balance between anabolism and catabolism.
- *Rasayan* therapy which regulates the circulation of vital fluid, eliminates the waste product, rejuvenate the nervous system as well.
- It prevents wasting of muscles, delays the ageing process, nourishes bones, tendons etc.. Prevents osteoporosis, prevents premature greying of hair and provides good sleep and appetite.
- *Rasayan* help keep body and mind function at their optimum best.

Probable action of *Rasayan* according to Modern view^[5]

Rasayan dravyas work as Immunomodulators and have antioxidant activity as well.

- In relation to non specific immunity these drugs increase activation of polymorphonuclear neutrophils (PMN) for phagocytosis and enhance their chemotactic capacity.
- In relation to specific immunity, they lead to proliferation of lymphocytes leading to production and also cytotoxic induction of T-helper and Natural Killer (NK) cells and activation of complement pathways. Also they significantly increase immunoglobulin levels.
- Immunostimulants offer promise in enhancing antigen specific (vaccine) and non-specific immune response against infections.
- *Achar Rasayan* acts as psycho immunomodulator, reduces stress and thus prevents release of free radicals and improves Psycho-Neuro Immunity (PNI)
- Till date a lot of research has been done on *Rasayan dravyas* for understanding their pharmacokinetics and pharmacodynamics and their probable mechanisms of action of various ailments. Majority of *Rasayan dravyas* have been claimed for their immunomodulatory effects via.^[6]
 - Modulation of cytokine secretion
 - Histamine release
 - Immunoglobulin secretion
 - Class switching
 - Cellular co-receptor expression
 - Lymphocyte expression
 - Phagocytosis

Hence the study was planned to evaluate the Immunomodulatory activity of *Shataputi Abhrak Bhasma* using modern Immunomodulatory assay.

AIMS AND OBJECTIVES

- *In-vitro* Immunomodulatory screening of *Shataputi Abhrak Bhasma* was carried out by means of Nitroblue Tetrazolium Dye Test. (NBT assay)

MATERIALS AND METHODS

The present experimental study was carried out in the Pharmacology Research Lab of the Pharmacology Department of Institute of Chemical Technology, Matunga, Mumbai.

1. Test Drug

Shataputi Abhrak Bhasma prepared using Electrical Muffle Furnace following *Rasa Jala Nidhi* reference.

2. Study Design

In-vitro Screening for Immunomodulatory activity using Nitroblue Tetrazolium Test (NBT) ^[7,8,9,10]

The NBT dye reduction test gives information about the phagocytic and intracellular killing functions of leucocytes which are necessary for normal microbicidal activity. The dye is taken into leucocytes by phagocytosis and then stimulation of the hexose monophosphate-shunt pathway (HMP) of glucose oxidation and concomitant changes in oxidative metabolism lead to the reduction of the dye to an insoluble blue crystalline form (formazan crystals). These blue crystals are visible in the light microscope and can be counted. The NBT test gives information about phagocytic function, since the dye is not taken into cells except by phagocytosis.

Chemicals and Reagents:

- Coli Endotoxin Standard: 20 ml of broth from each of S strains of E. Coli was boiled in water-bath for 2 hours, centrifuged at 2000g for 30 mins and the supernatant was pooled and stored -20°C.
- 0.15% Nitroblue tetrazolium dye: One part of 0.3% Nitroblue Tetrazolium (NBT) solution, prepared in 0.34% sucrose solution, was added to one part of phosphate buffer solution (PBS) and was used fresh.
- Leucocyte suspension: A suspension of leucocytes (5×10^6 /ml) was prepared in 0.5 ml PBS.
- Test compounds: *Shataputi Abhrak Bhasma* solutions were prepared in concentrations of 0.5, 1, 2.5, 5, 10 %. The solutions were centrifuged and the supernatants were used in the assay.

Procedure

A. Preparation of Leucocytes by Differential Lysis of Erythrocytes

Leucocytes were isolated by centrifugation after specific lysis of erythrocytes.

1. Anticoagulant added whole blood was well-suspended by gentle inversion and poured into a 50 ml conical tube.

2. 40 mL ice cold lysis buffer was added and mixed completely by gentle inversion.
3. It was kept on ice, mixed occasionally, until erythrocytes were lysed.
4. Centrifuged at 1,500 g for 5 minutes.
5. Supernatant was discarded by careful vacuum aspiration.
6. The leucocyte pellet was resuspended thoroughly in 1 mL ice cold lysis buffer by repeated pipetting.
7. After the cells have been homogeneously resuspended, additional 4 ml icecold lysis buffer was added, swirled gently to mix, and kept on ice for 10 minutes.
8. Cell suspension was then diluted to 50 mL with icecold 0.9% saline.
9. Mixed and centrifuged at 1,500 g for 5 minutes.
10. The supernatant was discarded by gentle vacuum aspiration. The leucocyte pellet resuspended thoroughly in 1 mL icecold 0.9% saline by repeated pipetting until the cell suspension was completely homogeneous, & then additional 9 ml ice cold 0.9% saline was added and swirled gently to mix.
11. Count the yield of cells on a Neubars chamber.

B. Nitroblue Tetrazolium (NBT) Dye Test

- The assay mixture consisted of 0.2 ml of 5×10^6 /ml of leucocyte suspension and 0.2-ml freshly prepared 0.15% NBT solution.
- 0.1 ml of test substance at different concentrations was added to the reaction mixture.
- 0.1 ml of endotoxin-activated plasma was added to the 0.15% NBT solution and leucocytes which served as a positive control (standard).
- A normal control was maintained in another test tube with leucocytes suspension, distilled water and NBT solution.
- All the test tubes were incubated separately at 37°C for 20 min and centrifuged gently at 400 g for 3-4 min. The supernatant was discarded.
- A drop of PBS was added and the cells were gently resuspended at the bottom of the test tube.
- A film was made by allowing a drop of this fluid to dry on a microscope slide.
- Slides were dried for 10-15 mins.
- Methanol fixation was carried out and again slides were kept for drying purpose.
- Slides were further stained in Giemsa stain for 15 mins and washed under tap water.
- After complete drying, the slides were observed under light microscope with oil immersion objective.

- 300 neutrophils were counted and the % of NBT positive cells containing the blue spots (stimulated) were determined.

OBSERVATIONS AND RESULTS

Table 1: Result for Immunomodulatory Effect (NBT Test)

S.No.	Test Substance	% NBT positive cells
1.	Normal Control	34.08
2.	Positive Control (Endotoxin activated plasma)	88
3.	0.5% <i>Shataputi Abhrak Bhasma</i>	75
4.	1% <i>Shataputi Abhrak Bhasma</i>	84
5.	2.5% <i>Shataputi Abhrak Bhasma</i>	84.4
6.	5% <i>Shataputi Abhrak Bhasma</i>	93
7.	10% <i>Shataputi Abhrak Bhasma</i>	93.5

DISCUSSIONS

- Nitroblue tetrazolium dye test is used to assess the Immunomodulatory activity of the test compound by determining its ability to stimulate the phagocytic activity in leucocytes.
- Once stimulated, the membrane permeable, water-soluble, yellow-colored, nitroblue tetrazolium is reduced to blue NBT formazan crystals by the leucocytes.
- The *Shataputi Abhrak Bhasma* stimulated phagocytic activity of the leucocytes in a concentration dependent manner as seen by the increased percentage of NBT positive cells.

CONCLUSION

- Thus *Shataputi Abhrak Bhasma*, exhibits a potent In-vitro Immunomodulatory (stimulant) activity in a concentration dependent manner.
- The results are self conclusive and indicate that *Shataputi Abhrak Bhasma* brings about stimulation of Leucocytes and thus in turn leads to highly significant phagocytic activity which is evident from the Invitro NBT test.
- The Immunomodulatory activity of *Shataputi Abhrak Bhasma* is concentration dependent. The activity of *Shataputi Abhrak Bhasma* at 5% and 10% is even higher than the positive control group (Endotoxin Activated Plasma). Thus it can be concluded that *Shataputi Abhrak Bhasma* which is highly rated as a *Rasayan* by various *Rasa Shastra Granthakars*, has a significant Invitro Immunomodulatory activity in concentration dependent manner. This also validates the principle that *Shataputi Abhrak Bhasma* acts as a *Rasayan* after subjecting to 100 *Putas*. Animal and Clinical trials shall further consolidate the above

results and shall help find mechanism of action of Immunomodulation of *Shataputi Abhrak Bhasma*.

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SHATAPUTI ABHRAK BHASMA PREPARATION



Raw Krushna Vajra Abhrak (Biotite)



Red Hot Stage of Nirvap



Abhrak after 1st Nirvap



Abhrak after 7th Nirvap



Dhanyabhrak Obtained



Chakrika in Sharav



Sharava Samputa



EMF at 980C-1st Puta



Abhrak Bhasma after 1st Puta



Abhrak Bhasma after 20 Putas

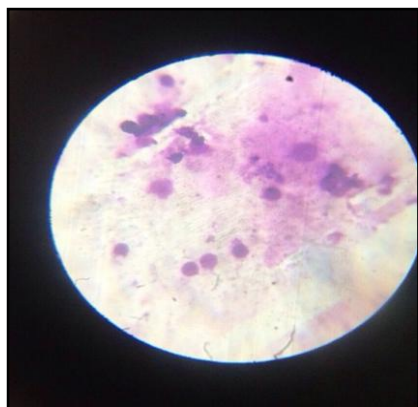


Abhrak Bhasma after 50 Putas

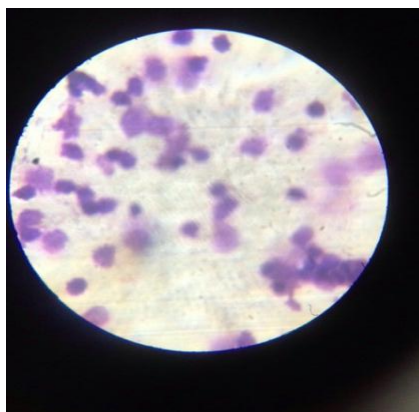


Abhrak Bhasma after 100 Putas

PHOTOGRAPHS OF INVITRO NBT ASSAY FOR IMMUNOMODULATORY ACTIVITY



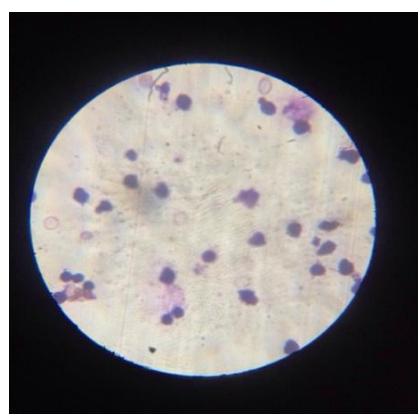
NBT-Control



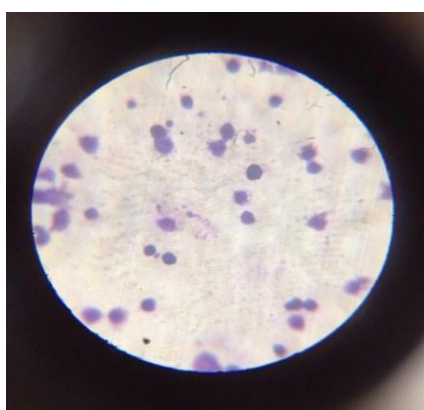
NBT- Positive Control



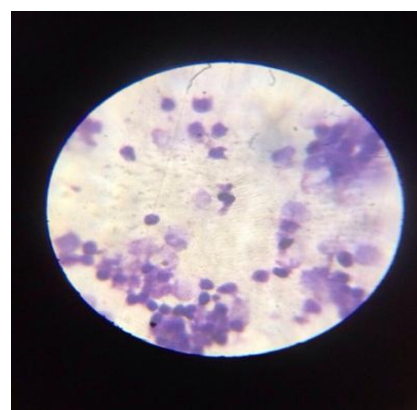
NBT-0.5% *Shataputi Abhrak Bhasma*



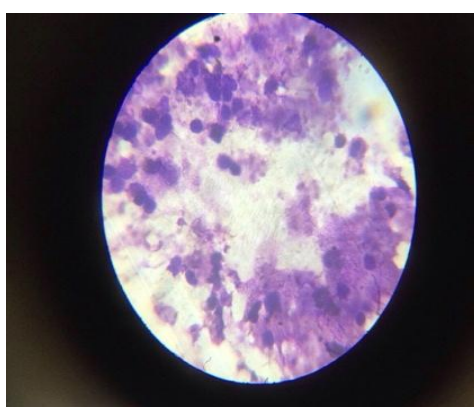
NBT-1% *Shataputi Abhrak Bhasma*



NBT-2.5% *Shataputi Abhrak Bhasma*



NBT-5% *Shataputi Abhrak Bhasma*



NBT-10% *Shataputi Abhrak Bhasma*